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M. Russell

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PATENTS ACT 1977

PATENTS FORM NO. 1/77 (Revised 1982)

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REQUEST FOR GRANT OF A PATENT

9009861.7

THE GRANT OF A PATENT IS REQUESTED BY THE UNDERSIGNED ON THE BASIS OF THE PRESENT APPLICATION

I Applicant's or Agent's reference (Please insert if available) CLB/AV101

II Title of invention CHEMICAL COMPOUNDS

III Applicant or Applicants (See note 2)

Name (First or only applicant) GLAXO GROUP LIMITED

Country United Kingdom State ADP Code No 0047 3587 001

Address Clarges House, 6-12 Clarges Street, London, W1Y 8DH, England

Name (of second applicant, if more than one)

Country State

Address

IV Inventor (see note 3)

(a) The applicant(s) is/are the
sole/joint inventor(s)

-or-

(b) A statement on Patents Form
No 7/77 is/will be furnished

V Name of Agent (if any) (See note 4)

C L Brewer

ADP CODE NO

3981990001

VI Address for Service (See note 5)

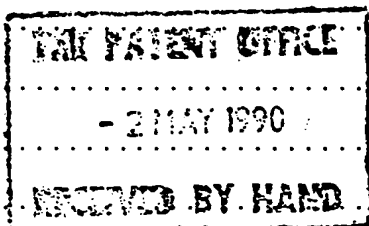
c/o Glaxo Holdings plc
63 Graham Street
London N1 8JZ

VII Declaration of Priority (See note 6)

Country

Filing date

File number



VIII The Application claims an earlier date under Section 8(3), 12(6), 15(4), or 37(4) (See note 7)

Earlier application or patent number and filing date

IX Check List (To be filled in by applicant or agent)

- | | |
|--|---|
| A The application contains the following number of sheet(s) | B The application as filed is accompanied by:- |
| 1 Request <u>1</u> Sheet(s) | 1 Priority document . |
| 2 Description <u>25</u> Sheet(s) | Translation of priority document |
| 3 Claim(s) Sheet(s) | 3 Request for Search..... |
| 4 Drawing(s) Sheet(s) | 4 Statement of Inventorship and Right to Grant..... |
| 5 Abstract Sheet(s) | |

X It is suggested that Figure No..... of the drawings (if any) should accompany the abstract when published.

XI Signature (See note 8)

NOTES:

C L Brewer

Agent for the Applicants

1. This form, when completed, should be brought or sent to the Patent Office together with the prescribed fee and two copies of the description of the invention, and of any drawings.

2. Enter the name and address of each applicant. Names of individuals should be indicated in full and the surname or family name should be underlined. The names of all partners in a firm must be given in full. Bodies corporate should be designated by their corporate name and the country of incorporation and, where appropriate, the state of incorporation within that country should be entered where provided. Full corporate details, eg a "corporation organised and existing under the laws of the State of Delaware, United States of America", trading styles, eg "trading as xyz company", nationality, and former names, eg "formerly (known as) ABC Ltd" are *not* required and should *not* be given. Also enter applicant(s) ADP Code No.(if known).

3. Where the applicant or applicants is/are the sole inventor or the joint inventors, the declaration (a) to that effect at IV should be completed, and the alternative statement (b) deleted. If, however, this is not the case the declaration (a) should be struck out and a statement will then be required to be filed upon Patent Form No 7/77.

4. If the applicant has appointed an agent to act on his behalf, the agent's name and the address of his place of business should be indicated in the spaces available at V and VI. Also insert agent's ADP Code No. (if known) in the box provided.

5. An address for service in the United Kingdom to which all documents may be sent must be stated at VI. It is recommended that a telephone number be provided if an agent is not appointed.

6. The declaration of priority at VII should state the date of the previous filing and the country in which it was made and indicate the file number, if available.

7. When an application is made by virtue of section 8(3), 12(6), 15(4) the appropriate section should be identified at VIII and the number of the earlier application or any patent granted thereon identified.

8. Attention is directed to rules 90 and 106 of the Patent Rules 1982.

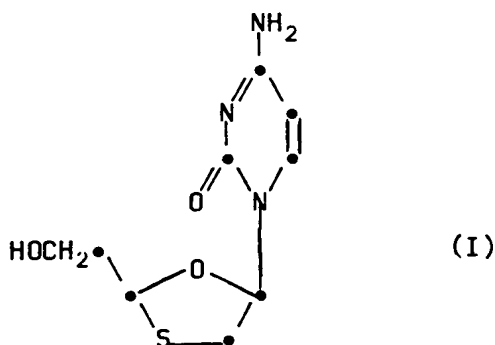
9. Attention of applicants is drawn to the desirability of avoiding publication of inventions relating to any article, material or device intended or adapted for use in war (Official Secrets Acts, 1911 and 1920). In addition after an application for a patent has been filed at the Patent Office the comptroller will consider whether publication or communication of the invention should be prohibited or restricted under section 22 of the Act and will inform the applicant if such prohibition is necessary.

10. Applicants resident in the United Kingdom are also reminded that, under the provisions of section 23 applications may not be filed abroad without written permission or unless an application has been filed not less than six weeks previously in the United Kingdom for a patent for the same invention and no direction prohibiting publication or communication has been given or any such direction has been received.

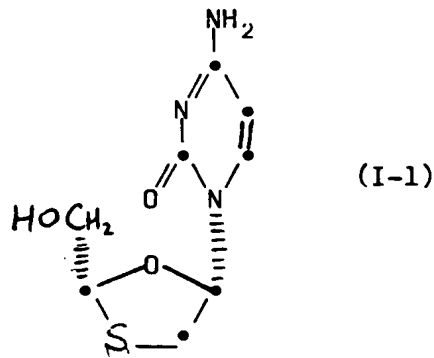
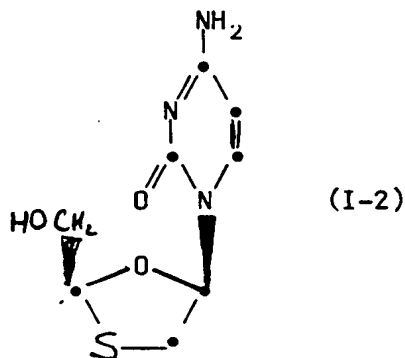
CHEMICAL COMPOUNDS

The present invention relates to nucleoside analogues and their use in medicine. More specifically the invention is concerned with 1,3-oxathiolane nucleoside analogues, pharmaceutical formulations thereof and the use in the treatment of viral infections.

The compound of formula (I)



also known as BCH-189 or NIGPB-21 has been described as having antiviral activity in particular against the human immunodeficiency viruses (HIV's), the causative agents of AIDS (5th Anti-Aids Conference, Montreal, Canada 5th-9th June 1989: Abstracts T.C.O.1 and M.C.P. 63). The compound of formula (I) is a racemic mixture of the two enantiomers of formulae (I-1) and (I-2):-



and was described and tested in the form of its racemate.

The only compound currently approved for the treatment of conditions caused by HIV is 3'-azido-3'-deoxythymidine (AZT, zidovudine, BW 509U). However, this compound has a significant side-effect liability and thus either cannot be employed or, once employed, may have to be withdrawn in a significant number of patients. There is in consequence a continuing need to provide compounds which are effective against HIV but with a concomitant significantly lower toxicity profile.

We have now found that, surprisingly, enantiomers of the compound of formula (I) are equipotent against HIV but that one of the enantiomers (the(-)-enantiomer) has considerably lower cytotoxicity than the other enantiomer. There is thus provided in a first aspect of the invention the (-) (or laevorotatory) enantiomer of the compound of formula (I) and pharmaceutically acceptable derivatives thereof.

The (-) enantiomer has the chemical name
(-)-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (hereinafter compound (A)). It is believed that it has the absolute stereochemistry of the compound of formula (I-1) which has the name (2R,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one.

Preferably Compound A is provided substantially free of the corresponding (+)-enantiomer, that is to say no more than about 5% w/w of the (+)- enantiomer, preferably no more than about 2%, in particular less than about 1% w/w is present.

By "a pharmaceutically acceptable derivative" is meant any pharmaceutically acceptable salt, ester, or salt of such ester, of the compound (A) or any other compound which, upon administration to the recipient, is capable of providing (directly or indirectly) the compound (A) or an antivirally active metabolite or residue thereof.

It will be appreciated by those skilled in the art that the compound (A) may be modified to provide pharmaceutically acceptable derivatives thereof, at functional groups in both the base moiety and at the hydroxymethyl group of the oxathiolane ring. Modification at all such functional groups are included within the scope of the

invention. However of particular interest are pharmaceutically acceptable derivatives obtained by modification of the 2-hydroxymethyl group of the oxathiolane ring.

Preferred esters of compound (A) include the compounds in which the hydrogen of the 2-hydroxymethyl group is replaced by an acyl function $R-\overset{\overset{O}{\parallel}}{C}$ in which the non-carbonyl moiety R of the ester is selected from hydrogen, straight or branched chain alkyl (e.g. methyl, ethyl, n-propyl, t-butyl, n-butyl), alkoxyalkyl (e.g. methoxymethyl), aralkyl (e.g. benzyl), aryloxyalkyl (e.g. phenoxymethyl), aryl (e.g. phenyl optionally substituted by halogen, C_{1-4} alkyl or C_{1-4} alkoxy); sulphonate esters such as alkyl- or aralkylsulphonyl (e.g. methanesulphonyl); amino acid esters (e.g. L-valyl or L-isoleucyl) and mono-, di- or tri-phosphate esters.

With regard to the above described esters, unless otherwise specified, any alkyl moiety present advantageously contains 1 to 16 carbon atoms, particularly 1 to 4 carbon atoms. Any aryl moiety present in such esters advantageously comprises a phenyl group.

In particular the esters may be a C_{1-16} alkyl ester, an unsubstituted benzyl ester or a benzyl ester substituted by at least one halogen (bromine, chlorine, fluorine or iodine), C_{1-6} alkyl, C_{1-6} alkoxy, nitro or trifluoromethyl groups.

Pharmaceutically acceptable salts of the compounds of formula (I) include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene-p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other acids such as oxalic, while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium), ammonium and NR_4^+ (where R is C_{1-4} alkyl) salts.

References hereinafter to a compound according to the invention includes both the compound (A) and its pharmaceutically acceptable derivatives.

The compounds of the invention either themselves possess antiviral activity and/or are metabolizable to such compounds. In particular these compounds are effective in inhibiting the replication of retroviruses, including human retroviruses such as human immunodeficiency viruses (HIV's), the causative agents of AIDS.

There is thus provided as a further aspect of the invention a compound (A) or a pharmaceutically acceptable derivative thereof for use as an active therapeutic agent in particular as an antiviral agent, for example in the treatment of retroviral infections.

In a further or alternative aspect there is provided a method for the treatment of a viral infection, in particular an infection caused by a retrovirus such as HIV, in a mammal including man comprising administration of an effective amount of a compound of (A) or a pharmaceutically acceptable derivative thereof.

There is also provided in a further or alternative aspect use of the compound (A) or a pharmaceutically acceptable derivative thereof for the manufacture of a medicament for the treatment of a viral infection.

The compounds of the invention are also useful in the treatment of AIDS related conditions such as AIDS-related complex (ARC), progressive generalised lymphadenopathy (PGL), AIDS-related neurological conditions (such as dementia or tropical paraparesis), anti-HIV antibody positive and HIV- positive conditions, Kaposi's sarcoma and thrombocytopenia purpurea.

The compounds of the invention are also useful in the prevention of progression to clinical illness of individuals who are anti-HIV antibody or HIV-antigen positive and in prophylaxis following exposure to HIV.

The compound (A) or pharmaceutically acceptable derivatives thereof, may also be used for the prevention of viral contamination of physiological fluids such as blood or semen in vitro.

It will be appreciated by those skilled in the art that reference herein to treatment extends to prophylaxis as well as the treatment of established infections or symptoms.

It will be further appreciated that the amount of a compound of the invention required for use in treatment will vary not only with the particular compound selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or veterinarian. In general however a suitable dose will be in the range of from about 0.1 to about 750mg/kg of bodyweight per day preferably in the range of 0.5 to 60 mg/kg/day, most preferably in the range of 1 to 20mg/kg/day.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example as two, three, four or more sub-doses per day.

The compound is conveniently administered in unit dosage form; for example containing 10 to 1500mg, conveniently 20 to 1000mg, most conveniently 50 to 700mg of active ingredient per unit dosage form.

Ideally the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 1 to about 75 μ M, preferably about 2 to 50 μ M, most preferably about 3 to about 30 μ M. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or orally administered as a bolus containing about 1 to about 100mg of the active ingredient. Desirable blood levels may be maintained by a continuous infusion to provide about 0.01 to about 5.0 mg/kg/hour or by intermittent infusions containing about 0.4 to about 15 mg/kg of the active ingredient.

While it is possible that, for use in therapy, a compound of the invention may be administered as the raw chemical it is preferable to present the active ingredient as a pharmaceutical formulation.

The invention thus further provides a pharmaceutical formulation comprising compound (A) or a pharmaceutically acceptable derivative thereof together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic

ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration or in a form suitable for administration by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active compound with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical formulations suitable for oral administration may conveniently be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution, a suspension or as an emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

The compounds according to the invention may also be formulated for parenteral administration (e.g. by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as

suspending, stabilising and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution, for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

For topical administration to the epidermis the compounds according to the invention may be formulated as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening agents, or colouring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising active ingredient in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the active compound with the softened or melted carrier(s) followed by chilling and shaping in moulds.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

For intra-nasal administration the compounds of the invention may be used as a liquid spray or dispersible powder or in the form of drops.

Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilising agents or

suspending agents. Liquid sprays are conveniently delivered from pressurised packs.

For administration by inhalation the compounds according to the invention are conveniently delivered from an insufflator, nebuliser or a pressurised pack or other convenient means of delivering an aerosol spray. Pressurised packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurised aerosol the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compounds according to the invention may take the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges or e.g. gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator.

When desired the above described formulations adapted to give sustained release of the active ingredient may be employed.

The pharmaceutical compositions according to the invention may also contain other active ingredients such as antimicrobial agents, or preservatives.

The compounds of the invention may also be used in combination with other therapeutic agents for example other antiinfective agents. In particular the compounds of the invention may be employed together with known antiviral agents.

The invention thus provides, in a further aspect, a combination comprising the compound (A) or a physiologically acceptable derivative thereof together with another therapeutically active agent, in particular an antiviral agent.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier therefor comprise a further aspect of the invention.

Suitable therapeutic agents for use in such combinations include acyclic nucleosides such as aciclovir or gancyclovir, interferons such as α , β or γ -interferon, renal excretion inhibitors such as probenecid, nucleoside transport inhibitors such as dipyridamole, 2',3'-dideoxynucleosides such as AZT, 2',3'-dideoxycytidine, 2',3'-dideoxyadenosine, 2',3'-dideoxyinosine, 2',3'-dideoxythymidine, 2',3'-dideoxy-2',3'-didehydrothymidine and 2',3'-dideoxy-2',3'-didehydrocytidine, immunomodulators such as interleukin II (IL2) and granulocyte macrophage colony stimulating factor (GM-CSF), erythropoetin, ampliten, thymomodulin, thymopentin, foscarnet, ribavirin and inhibitors of HIV binding to CD4 receptors e.g. soluble CD4, CD4 fragments, CD4 hybrid molecules, glycosylation inhibitors such as 2-deoxy-D-glucose, castanospermine and 1-deoxynojirimycin.

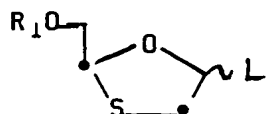
The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

When the compound (A) or a pharmaceutically acceptable derivative thereof is used in combination with a second therapeutic agent active against the same virus the dose of each compound may be either the same as or differ from that when the compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

The compound (A) and its pharmaceutically acceptable derivatives may be prepared by any method known in the art for the preparation of compounds of analogous structure.

It will be appreciated by those skilled in the art that for certain of the methods described herein below the desired stereochemistry of the compound (A) may be obtained either by commencing with a optically pure starting material or by resolving the racemic mixture at any convenient stage in the synthesis. In the case of all the processes the optically pure desired product may be obtained by resolution of the end product of each reaction

In one such process (A) a 1,3-oxathiolane of formula (VIII)

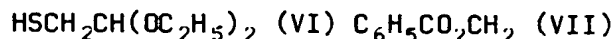


(VIII)

wherein the anomeric group L is a displaceable group, is reacted with an appropriate base. Suitable groups L include -OR where R is an alkyl group, e.g. a C₁₋₆alkyl group such as methyl or R is an acyl group, e.g. a C₁₋₆alkyl group such as acetyl or halogen, for example iodine, bromine or chlorine.

The compound of formula VIII is conveniently reacted with cystidine or an appropriate pyrimidine base precursor thereof (previously silylated with a silylating agent such as hexamethyldisilazane) in a compatible solvent such as methylene chloride using a Lewis acid such as titanium tetrachloride or trimethylsilyltriflate.

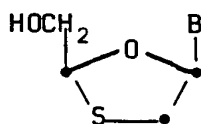
The 1,3-oxathiolanes of formula (VIII) may be prepared for example by reaction of an aldehyde of formula (VII) with a mercaptoacetal of formula (VI) in a compatible organic solvent, such as toluene in the presence of an acid catalyst for example a Lewis acid such as zinc chloride.



The mercaptoacetals of formula (VI) may be prepared by methods known in the art, for example G. Hesse and I. Jorder, Chem. Ber 85, 924-932 (1952).

The aldehydes of formula (VII) may be prepared by methods known in the art for example E. G. Halloquist and H. Hibbert, Can. J. Research, 8, 129-136 (1933).

In a second process (B) the compound (A) is obtained by base interconversion of a compound of formula (IX)

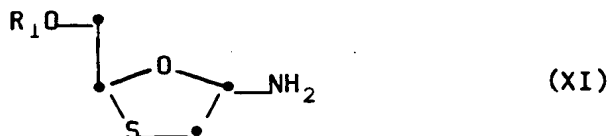


(IX)

where B is a base convertible to cytosine

Such interconversion may be effected either by simple chemical transformation (e.g. the conversion of uracil base to cytosine) or by an enzymatic conversion using a deoxyriboxyl transferase. Such methods and conditions for base interconversion are well known in the art of nucleoside chemistry.

In a third process (C) a compound of formula (XI)



may be converted to the compound (A) by conversion of the anomeric NH_2 group to the cytosine base by methods well known in the nucleoside chemistry art.

Many of the reactions described hereinabove have been extensively reported in the context of nucleoside synthesis, for example in Nucleoside Analogs - Chemistry, Biology and Medical Applications, R. T. Walker et. al., eds, Plenum Press, New York (1979) at pages 165-192; T. Ueda in Chemistry of Nucleosides and Nucleotides, Vol I, L B Townsebd ed., Plenum Press, New York (1988) at pages 165-192 the disclosures of which are incorporated by reference herein.

It will be appreciated that the above reactions may require the use of, or conveniently may be applied to, starting materials having protected functional groups, and deprotection might thus be required as an intermediate or final step to yield the desired compound. Protection and deprotection of functional groups may be effected using conventional means. Thus, for example, amino groups may be protected

by a group selected from aralkyl (e.g. benzyl), acyl or aryl (e.g. 2,4-dinitrophenyl); subsequent removal of the protecting group being effected when desired by hydrolysis or hydrogenolysis as appropriate using standard conditions. Hydroxyl groups may be protected using any conventional hydroxyl protecting group, for example, as described in 'Protective Groups in Organic Chemistry', Ed. J. F. W. McOmie (Plenum Press, 1973) or 'Protective Groups in Organic Synthesis' by Theodora

W. Greene (John Wiley and Sons, 1981). Examples of suitable hydroxyl protecting groups include groups selected from alkyl (e.g. methyl, t-butyl or methoxymethyl), aralkyl (e.g. benzyl, diphenylmethyl or triphenylmethyl), heterocyclic groups such as tetrahydropyranyl, acyl (e.g. acetyl or benzoyl) and silyl groups such as trialkylsilyl (e.g. t-butyldimethylsilyl). The hydroxyl protecting groups may be removed by conventional techniques. Thus, for example, alkyl, silyl, acyl and heterocyclic groups may be removed by solvolysis, e.g. by hydrolysis under acidic or basic conditions. Aralkyl groups such as triphenylmethyl may similarly be removed by solvolysis, e.g. by hydrolysis under acidic conditions. Aralkyl groups such as benzyl may be cleaved for example by treatment with BF_3 /etherate and acetic anhydride followed by removal of acetate groups so formed at an appropriate stage in the synthesis. Silyl groups may also conveniently be removed using a source of fluoride ions such as tetra-n-butylammonium fluoride.

In the above processes the compounds of formula (I) are generally obtained as a mixture of the cis and trans isomers of which the cis isomers are the compounds of interest.

These isomers may be separated by physical means e.g. chromatography on silica gel or by fractional crystallisation, either directly or on a suitable derivatives thereof, eg acetates (prepared for example with acetic anhydride) followed, after separation, by conversion back to the parent product (eg by deacetylation with methanolic ammonia).

Pharmaceutically acceptable salts of the compounds of the invention may be prepared as described in US Patent No. 4,383,114, the disclosure of which is incorporated by reference herein. Thus, for example, when it is desired to prepare an acid addition salt of compound (A) the product of any of the above procedures may be converted into a salt by treatment of the resulting free base with a suitable acid using conventional methods. Pharmaceutically acceptable acid addition salts may be prepared by reacting the free base with an appropriate acid optionally in the presence of a suitable solvent such as an ester (e.g. ethyl acetate) or an alcohol (e.g. methanol, ethanol or isopropanol). Inorganic basic salts may be prepared by

reacting the free base with a suitable base such as an alkoxide (e.g. sodium methoxide) optionally in the presence of a solvent such as an alcohol (e.g. methanol). Pharmaceutically acceptable salts may also be prepared from other salts, including other pharmaceutically acceptable salts, of the compound (A) using conventional methods.

Compound (A) may be converted into a pharmaceutically acceptable phosphate or other ester by reaction with a phosphorylating agent, such as POCl_3 , or a suitable esterifying agent, such as an acid halide or anhydride, as appropriate. An ester or salt of compound of (A) may be converted to the parent compound for example by hydrolysis.

Resolution of the final product, or an intermediate or starting material therefor may be effected by any suitable method known in the art : see for example 'Stereochemistry of Carbon Compounds' by E. L. Eliel (McGraw Hill, 1962) and 'Tables of Resolving Agents' by S. H. Wilen.

Thus for example the compound (A) may obtained by chiral HPLC using a suitable stationary phase for example acetylated β -cyclodextrin or cellulose triacetate and a suitable solvent for example an alcohol such as ethanol or an aqueous solution of for example tri ethyl ammonium acetate. Alternatively the compounds may be resolved by enzyme mediated enantioselective catabolism with a suitable enzyme such as cytidine deaminase or selective enzymatic degradation of a suitable derivative using a 5'-nucleotidase.

The invention will be further described by the following examples which are not intended to limit the invention in any way. All temperatures are in degrees Celcius.

INTERMEDIATE 1

5-Methoxy-1,3-oxathiolane-2-methanol, benzoate.

A solution of zinc chloride (1.6g) in hot methanol (15ml) was added to a stirred solution of mercaptoacetaldehyde, dimethyl acetal (34.2g) and benzoyloxy acetaldehyde (48.3g) in toluene (1300ml) which was then heated to reflux under nitrogen for 50 min. The cooled mixture was concentrated, diluted with some toluene, then filtered through

Kieselguhr. The combined filtrates and toluene were washed with aqueous saturated sodium bicarbonate solution (X2) and brine, dried (MgSO_4) then evaporated to an oil which was subjected to column chromatography on silica (2kg, merck 9385) eluted with chloroform to give the title product as an oil (45.1g) a mixture of anomers (ca 1:1); ^1H NMR ($\text{DMSO}-d_6$) 3.1-3.3(4H), 3.42(6H), 4.4-4.6 (4H), 5.41(1H), 5.46 (1H), 5.54 (1H), 5.63 (1H), 7.46 (4H), 7.58 (2H), 8.07 (4H); γ_{max} (CHBr_3) 1717.6 cm^{-1} .

INTERMEDIATE 2

(\pm)-cis-1-(2-benzoyloxymethyl-1,3-oxathiolan-5-yl)
-(1H)-pyrimidin-2-4-dione

A mixture of finely ground uracil (9.62g) hexamethyl disilazane (50 ml) and ammonium sulphate (30 mg) was heated at reflux under nitrogen until a clear solution was obtained. This was cooled and then evaporated to a colourless oil, which was dissolved, under nitrogen atmosphere, in acetonitrile (100ml). The solution was added to a stirred ice cooled solution of 5-methoxy-1,3-oxathiolane-2-methanol, benzoate (intermediate 1) (19.43g), in acetonitrile (600ml) and trimethyl silyl trifluoromethanesulphonate (14.7ml) was added. The ice bath was removed, and the solution was heated at reflux under nitrogen for 45 mins. After cooling and evaporation, the residue was purified by column chromatography over 1kg of silica gel (Merck 9385) eluting with chloroform/methanol 9:1. Appropriate fractions were cooled and evaporated to afford a crude residue. This was fractionally crystallized from the minimum of hot methanol (c.1200ml) to afford the title compound (6.32g) as white crystals. ^1H NMR ($d_6\text{DMSO}$) δ 11.36 (1H,bs). 7.50-8.00 (6H,m), 6.20 (1H,t), 5.46 (2H,m), 4.62 (2H, m), 3.48 (1H, m), 3.25 (1H, m).

INTERMEDIATE 3

(\pm)-(Cis)-4-amino-1-(2-benzoyloxymethyl-1,3-oxathiolan-5-yl)-(1H)-
pyrimidin-2-one

Method (a)

A suspension of cytosine (20.705g) and ammonium sulphate (few mgs) in hexamethyldisilazane (110ml) was stirred and heated at reflux for 2½h, under nitrogen. Solvent was removed by evaporation, and the residual solid was dissolved in dry acetonitrile (350ml). This solution was transferred using flexible needle techniques into a stirred, ice-chilled solution of 5-methoxy-1,3-oxathiolane-2-methanol, benzoate (Intermediate I) (43.57g) in acetonitrile (650ml) under nitrogen. Trimethylsilyl trifluoromethanesulphonate (33ml) was added, then the solution was allowed to warm to ambient temperature (1½h) then heated to reflux for an overnight period. The residue mixture was concentrated, diluted with saturated aqueous sodium bicarbonate solution (500ml), then extracted with ethyl acetate (3x500ml). The combined extracts were washed with water (2x250ml) and brine (250ml) dried (MgSO₄) then evaporated to a foam which was subjected to column chromatography on silica (600g, merck 7734), eluted with ethyl acetate-methanol mixtures to give a mixture of anomers (ca 1:1 31.59g). The mixture was crystallised from water (45ml) and ethanol (9.0ml) to give a solid (10.23g) which was recrystallised from ethanol (120ml) and water (30ml) to give the title product as a white solid (9.26g); λ_{max} (MeOH) 229.4mm (E_{1cm}^{1%} 610); 272.4mm (E_{1cm}^{1%} 293); ¹H NMR (DMSO d₆) δ 3.14 (1H), 3.50 (1H), 4.07 (2H), 5.52 (1H), 5.66 (1H), 6.28 (1H), 7.22 (2H), 7.56 (2H), 7.72 (2H), 8.10 (2H).

Method (b)

Phosphorous oxychloride (7.0ml) was added dropwise to a stirred, ice-cooled suspension of 1,2,4-triazole (11.65g) in acetonitrile (120ml) then, keeping the internal temperature below 15°C, triethylamine (22.7ml) was added dropwise. After 10 min a solution of (±)-cis -1-(2-benzoyloxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2,4-dione. (Intermediate 2) (6.27g) in acetonitrile (330ml) was slowly added. Stirring was then continued at room temperature overnight. The mixture was added by means of an ice bath and triethylamine (30ml) was slowly cooled followed by water (21ml). The resultant solution was evaporated, and the residue was partitioned between saturated sodium bicarbonate solution (400ml) and chloroform

(3x200ml). The combined chloroform extracts were dried and magnesium sulphate, filtered and evaporated to give a crude residue (9.7g). The residue was dissolved in 1,4-dioxan (240ml) and concentrated aqueous ammonia solution (s.g 0.880, 50ml) was added. After 1½h the solution was evaporated and the residue dissolved in methanol. This caused precipitation of a solid, which was filtered off. The mother liquors were purified by column chromatography over silica gel (Merck 9385, 600g). Appropriate fractions were pooled and evaporated to give the title compound as a fawn solid (2.18g), identical to that obtained by Method (a).

Example 1

(±)-(Cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one

A suspension of (Cis)-4-amino-1-(2-benzoyloxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (Intermediate 3) (8.19g) and Amberlite IRA-400 (OH) resin (8.24g) in methanol (250ml) was stirred and heated to reflux for 1½h. Solids were removed by filtration then washed with methanol. The combined filtrates were evaporated. The residue was triturated with ethyl acetate (80ml). The resulting white solid was collected by filtration to give the title product (5.09g), 1H NMR (DMSO-d₆) 3.04 (1H), 3.40 (1H), 3.73 (2H), 5.18 (1H), 5.29 (1H), 5.73 (1H), 6.21 (1H), 7.19 (2H), 7.81 (1H).

Example 2: Chiral HPLC separation of the enantiomers of

(±)-(Cis)-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolane 5-yl)-(1H)-pyrimidin-2-one.

(A) The racemic product of Example 1 (25mg) was subjected to preparative HPLC under the following conditions:

Column:	Merck Hibar cellulose triacetate, 250 x 10mm, 10μ
Eluant:	ethanol;
Flow:	3ml/min;
Detection:	uv, 270nm;

Temperature: ambient.

Evaporation of the appropriate pooled fractions gave (2R,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (6.8mg, e.e. ca 100%) and (2S,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (3.6mg, e.e. ca 90%).

(B) The racemic product of Example 1 (26mg) was subjected to preparative HPLC under the following conditions:

Column: ASTEC cyclobond I acetyl, 250x4.6mm;
Eluant: 0.2% triethylammonium acetate (prepared by adding glacial acetic acid to 0.2% triethylamine in water, to a final pH 7.2);
Flow: 2ml/min;
Detection: uv, 300nm;
Temperature: ambient.

Evaporation of appropriate fractions gave crude (2R,Cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)pyrimidin-2-one (25mg) and crude (2S,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (17mg). These fractions were separately subjected to further preparative HPLC run under the following conditions:

Column: ASTEC cyclobond I acetyl, 250x4.6mm
Eluent: 15mM ammonium acetate,, pH 6.8;
Flow: 0.5ml/min
Detection: uv, 300nm;
Temperature: 5⁰.

Evaporation of the appropriate pooled fractions gave (2R, Cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (5.0mg, e.e ca. 91%) and (2S,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (7.6mg, e.e. ca 96%).

Example 3

(-)-Cis--4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one

(i) (±)-Cis 4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidinone, 6'-dihydrogenphosphate, ammonium salt

A cold (0°), stirred suspension of (±)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (1.00g) (Example 1) in dry trimethylphosphate (20ml) was treated with phosphorous oxychloride (2.44ml), and the mixture was stirred at 0° for 35 mins then quenched in ice water (60g). The cold mixture was adjusted to pH 2.5 by the addition of aqueous N-sodium hydroxide, then applied to a charcoal column (10g, DARCO), which was eluted with water, then ethanol-aqueous ammonia. Fractions containing crude monophosphate were combined and concentrated. The resulting solution was applied to a column containing 25g of DEAE Sephadex A-25 (HCO₃⁻ form). Elution was performed with a gradient of water (120ml) to 0.1 M-NH₄HCO₃ (240ml), then 0.2, 0.3 and 4M NH₄HCO₃ (120, 240, 400ml respectively). Appropriate fractions were combined and concentrated. The residual solution was diluted with water (40ml) and freeze-dried to give the title product as a white solid (1.37g); λ_{max} (pH6 buffer), 271.0nm (E_{1%}_{1cm} 190); ¹H NMR (D₂O) δ 3.23 (1H), 3.55 (1H), 4.0-4.2 (2H), 5.43 (1H), 6.07 (1H), 6.33 (1H), 8.08 (1H).

(ii) (2R, Cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5yl)-(1H)-pyrimidin-2-one and (2S, cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one

5'-Nucleotidase (from crotalus atrox venom) [EC 3.1.3.5] (60mg at 17 units/mg) was added to a solution of (±)-cis-4-amino-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one, 6'-dihydrogenphosphate, ammonium salt (1.35g) in buffer [30ml, prepared from glycine (526mg) and magnesium chloride (190mg) in water (100ml)] and the mixture was incubated at 37° for 2.5h. More enzyme (20mg) was added, and incubation was continued for a further 3.5h. The resulting mixture was applied to a column of DEAE Sephadex

A-25(HCO₃⁻form). Elution was performed with water (160ml), then 0.1, 0.2, 0.3 and 0.4M NH₄HCO₃ (200ml each). Appropriate fractions containing the first eluted component were combined and evaporated, the residue was subjected to column chromatography on silica (60g, Merck 7734), eluted with chloroform-methanol mixtures. Evaporation of the appropriate fractions from methanol-ethyl acetate gave (2R, Cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one as a white solid (0.30g)- [α]_D²¹+137° (c.1.04 MeOH); ¹H NMR (DMSO) δ 3.04 (1H), 3.40 (1H), 3.73 (2H), 5.18 (1H), 5.29 (1H), 5.73 (1H), 6.21 (1H), 7.19 (2H), 7.81 (1H).

Appropriate fractions from the Sephadex column, containing the second eluted component, were combined and evaporated. The residue was dissolved in water (30ml), treated with alkaline phosphatase (from *Escherichia coli*) [EC 3.1.3.1] (1.5ml at 416units/ml), then incubated at 37° for one hour. Solvent was removed by evaporation and the residue was subjected to column chromatography on silica (60g, Merck 7734), eluted with chloroform-methanol mixtures. Evaporation of the appropriate fractions from methanol-ethyl acetate gave (2S,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one as a white solid (0.32g); [α]_D²¹-132° (c.1.08, MeOH); ¹H NMR (DMSO) δ 3.04 (1H), 3.40 (1H), 3.73 (2H), 5.18 (1H), 5.29 (1H), 5.73 (1H), 6.21 (1H), 7.19 (2H), 7.81 (1H).

Example 4.

(-)-Cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H) pyrimidin-2-one

(i) Three 50ml flasks of nutrient broth (Oxoid Ltd) were inoculated with a loopful each of *Escherichia coli* (ATCC 23848) scraped from a Nutrient Agar plate. The flasks were incubated overnight at 37°C with shaking at 250 rev/min and then each flask was used to inoculate 4l of CDD medium (glutamic acid, 3g/l; MgSO₄, 0.2g/l; K₂SO₄, 2.5g/l; NaCl, 2.3g/l, Na₂HPO₄·2H₂O, 1.1g/l, NaH₂PO₄·2H₂O 0.6g/l cytidine, 1.2g/l) in a seven litre fermenter. The cultures were fermented at 750 rev/min, 37°C with aeration at 4l/min. After growth for 24hrs the cells were collected by centrifugation (5000g, 30

minutes) to yield 72g wet weight. The cell pellet was resuspended in 300ml of 20mM Tris HCl buffer (pH 7.5) and disrupted by sonication (4 x 45 seconds). The cell debris was removed by centrifugation (30,000 g, 30 minutes) and the protein in the supernatant was precipitated by addition of ammonium sulphate to 75% saturation. The precipitate was collected by centrifugation (30,000g. 30 minutes) and the pellet was resuspended in 25ml of HEPES buffer (100mM, pH 7.0) containing ammonium sulphate (75% saturation).

Enzyme solution was prepared by centrifugation at 12,000 rpm for 30 mins. The supernatant was discarded and the pellet dissolved in Tris HCl buffer (pH 7.0; 100mM) to the original volume.

(ii) The product of Example 1 (115mg) was dissolved in water (100ml), and stirred. Enzyme solution (0.5ml) was added, and the mixture was maintained at a constant pH by the continual addition of HCl (25mM). The conversion was monitored by chiral HPLC, which showed that the (+) enantiomer of the substrate was preferentially deaminated. After 22hr the (+) enantiomer of the substrate (RT 12.5min) had been completely removed, and the solution was adjusted to pH 10.5 by the addition of conc. sodium hydroxide.

The solution produced above was eluted through a column QAE Sephadex (A25; Pharmacia; 30X 1.6cm), pre-equilibrated to pH11. The column was washed with water (200ml) and then with HCl (0.1M). Fractions (40ml) were taken, and analysed by reversed phase HPLC. Fractions 5-13, containing the unreacted (-) enantiomer of the substrate, were combined and adjusted to pH 7.5 with HCl.

Fraction 47, containing deaminated product, was adjusted to pH7.5 with dil. NaOH. Analysis by chiral HPLC showed that this material was a mixture, consisting of one enantiomer (RT 10.2min) as the major component with the other enantiomer (RT 8.5min) as a minor component (e.e ca 90%).

(iii) Stage (ii) above was repeated on a larger scale. The compound of Example 1 (363mg) in 250ml of water was incubated with enzyme solution (0.5ml), prepared as in Stage (i). Further aliquots (0.5ml)

of enzyme were added after 18 and 47 hrs. The reaction mixture was stirred for 70hr., then left standing for a further 64hr. Analysis by chiral hplc indicated that the (+) enantiomer of the substrate had been completely deaminated, and the resulting solution was adjusted to pH10.5 with NaOH.

The solution above was loaded onto the same QAE column, and eluted as in stage (i) Fractions 2-6, containing a mixture of the residual substrate and deaminated product, were bulked. Fractions 7-13, containing the residual substrate ((-) enantiomer), were bulked and adjusted to pH7.5. Fractions 25-26, containing deaminated product, were bulked and neutralised.

Fractions 2-6 above were re-eluted through the same QAE column. Fractions 3-11 from this second column contained unrected substrate ((-) enantiomer). Fraction 70 contained the deaminated product.

(iv) The resolved substrate fractions from stage (ii) and (iii) were combined and adjusted to pH7.5. This solution was eluted through a column of XAD-16 (40x2.4cm), packed in water. The column was washed with water, and then eluted with acetone: water (1:4 v/v). Fractions containing the (-) enantiomer of BCH 189 were bulked and freeze-dried to give a white powder (190mg, B1916/41/1).

The HPLC methods used above were as follows:-

1. Reversed Phase analytical HPLC

Column	:	Capital Cartridge Spherisorb ODS-2 (5uM) 150x4.6mm
Eluant	:	Ammonium dihydrogen phosphate (50mM)+ 5% MeCN
Flow	:	1.5ml/min
Detection	:	UV, 270nm
Retention Times	:	BCH 189 5.5min deaminated BCH -189 8.1min

2. Chiral analytical HPLC

Column : Cyclobond I Acetyl
250x4.6mm
Eluant : 0.2% Triethylammonium acetate (pH7.2)
Flow : 1.0ml/min
Detection : UV, 270nm
Retention Times : BCH 189 11.0 and 12.5min
: deaminated BCH-189 8.5 and 10.2 min
(The bioconversion was followed by monitoring the loss of the peak at 12.5min.; and accumulated of product at 10.2min).

Example 5- Biological Activity

Antiviral Activity

Antiviral activity of the compounds of Example 2 were determined against three strains of HIV-1 and one strain of HIV-2 in the following cell lines.

JM cells, a semi-nature T-cell line derived from a patient with lymphoblastic leukaemia, infected with HIV-1 strain GB8.

C8166 cells, a human umbilical cord blood lymphocyte cell line, infected with HIV-1-strain RF.

MT-4 cells, a human T-cell leukaemia cell line, infected with HIV-1 stain RF.

CEM cells, a human T lymphoblastoid cell line, infected with HIV-1 strains RF and U455, or HIV-2 strain ROD.

Antiviral activities in C8166, JM, and CEM cells were determined by inhibition of syncytium formation (Tochikura et al Virology, 164, 542-546) and in MT-4 cells by inhibition of formazan conversion (Baba et al; (1987) Biochem Biophys Res Commun., 142, 128-134; Mossman (1983) J.Immun Meth; 65, 55-57). Antiviral activities were also determined by analysing the amount of HIV p24 antigen synthesised in the presence and absence of enantiomers.

The results are shown in Tables 1 and 2 below:-

Table 1

	50% Antiviral Activity ($\mu\text{g/ml}$)					
Assay	Formazan	inhibition of syncytium formation				
Cells	MT-4	CEM	CEM	CEM	JM	C8166
Virus	HIV-1 RF	HIV-2-ROD	HIV-1 RF	HIV-1 U455	HIV-1GB8	HIV-1 RF
(+)-enantiomer	0.28	0.045	0.07	0.006	0.03	0.05
(-)-enantiomer	0.20	0.055	0.04	0.008	0.05	0.01

Table 2

Inhibition of HIV p24 Synthesis

50% Inhibition HIV p24 Synthesis ($\mu\text{g/ml}$)			
Cells	C8166	JM	MT-4
Virus	RF	GB8	RF
(+)-enantiomer	0.021	0.033	0.0008
(-)-enantiomer	0.016	0.016	0.0004

B Cytotoxicity

The cytotoxicities of the compounds of Example 2, the racemic compound (BCH-189; Example 1) and a 50/50 mixture of the two enantiomers were determined in five CD4 cell lines: H9, JM, CEM, C8166 and U937.

Compounds for test were serially diluted from $100\mu\text{g/ml}$ to $0.3\mu\text{g/ml}$ (final concentrations) in 96 well microtitre plates. 3.6×10^4 cells were inoculated into each well of the plates including drug-free controls. After incubation at 37°C for 5 days, the viable cell count was determined by removing a sample of cell suspension and counting Trypan Blue excluding cells in a haemocytometer.

The results are shown in Table 3.

Table 3

Cytotoxicity

	50% Cytotoxicity ($\mu\text{g/ml}$)				
Compound	CEM cells	JM cells	H9 cells	U937 cells	C8166 cells
(+)-enantiomer	1	1.5	2	4	35
(-)-enantiomer	>100	30	>100	>100	>100
BCH-189	3	3.5	8	15	90
1:1 Mix of enantiomers	2.5	ND*	ND	ND	ND

*ND = Not Determined